Morphometric diversity of indigenous Honeybees, *Apis mellifera* (Linnaeus, 1758), in Saudi Arabia

(Insecta: Apidae)

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Abstract. Twenty four morphological traits of Honeybees (*Apis mellifera* Linnaeus, 1758) were evaluated in 198 native colonies within Saudi Arabia to differentiate among populations. Principal component analysis based on colony means and k-means clustering proposed a separation of Saudi Honeybees into three clusters. These were confirmed by discriminant analysis, which reclassified colonies with 100% accuracy into clusters two and three and 96% accuracy into cluster one. Results indicate significant morphometric variation and a cline of factor one (characters associated with body size) from the north (cluster one) to the south (cluster three), with the highest dissimilarities between bees from the far north and the far south. The substantial variation detected in this study supported the previous description of Saudi Arabian Honeybees made by RUTTNER in 1976, which, based on few samples, was not representative of this large and diverse country.

Key words. Yemeni Honeybee, Saudi Arabia, morphometry, clinal transition, discriminanat analysis.

Introduction

The Honeybee *Apis mellifera* (Linnaeus, 1761) is naturally spread in Europe, Africa and Western Asia (MIGUEL et al. 2011). Based on morphometry, 26 subspecies have been identified and clustered into four evolutionary lineages (RUTTNER 1988, SHEPPARD et al. 1997, ENGEL 1999, SHEPPARD & MEIXNER 2003, MIGUEL et al. 2011). In Saudi Arabia, *Apis mellifera* exists throughout the country, especially in the southern and western regions next to the Red Sea coast, from Tabouk (29°00'N, 37°20'E) in the north near the Jordan border to Jazan (16°54'N, 42°35'E) in the south-west near the Yemen border. Within this large area, most of the Honeybee colonies (approximately 80%) are still kept in traditional wooden-log hives and occupy varied ecological niches, from deserts to arid subtropical areas including mountainous regions in the south (ALQARNI et al. 2011). Towards the far south of Yemen, *Apis mellifera jemenitica* (Ruttner, 1975) has been reported, and apparently shares similar characteristics with the native bees of Saudi Arabia. Towards the far north, a closer connection with *Apis mellifera syriaca* (Skorikov, 1929) may exist. Previous work by RUTTNER (1976) has described *Apis mellifera jemenitica* as an indigenous bee race of Saudi Arabia, Yemen and Oman, in zones with very high temperature and low precipitation. In that work, RUTTNER included only a few bee samples from three locations within Saudi Arabia: Sabya (17°15'N, 42°62'E), Riyadh (24°64'N, 46°77'E) and AIhasa (25°33'N, 49°63'E). He described the smallest *A. m. jemenitica* from these locations according to the most popular taxonomic traits used in the analysis of bee races. However, reference data are available only for the two samples from Sabya (RUTTNER et al. 1978, GROMISZ 1981).

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Fig. 1. Outline map of Saudi Arabia that shows the locations where bee samples have been collected (n=36). A total of 198 bee colonies were sampled: along the Red Sea coast (n=32: Tabouk, Al-Madinah, Al-Taif, Asir, Al-Baha, Jazan and Najran) and middle areas (n=4: Al-Jouf, Hail, Al-Qaseem and Riyadh).

Although RUTTNER'S work recognized the native bee race of Saudi Arabia, it was not possible to report on variations between different populations within the country. ALQARNI (1995) reported significant biological and morphological differences between and within the native Saudi Honeybee populations sampled from Saudi Arabia's Abha region. However, a thorough analysis of morphometic variation of the native bee populations within Saudi Arabia and its relation to other bee races is still needed. Such research is urgent, as the structure and distribution of the native bee race is endangered by irreversible hybridization due to substantial importations of other bee races from neighbouring countries. In this paper we investigate the morphological variation of the native Honeybee within Saudi Arabia.

Material and methods

Samples of bees with 10 workers each were taken from 198 local colonies at 36 locations within 11 beekeeping areas, most of them located next to the Red Sea coast (n=32: Tabouk, Al-Madinah, Al-Taif, Asir, Al-Baha, Jazan and Najran) and in the middle regions (n=4: Al-Jouf, Hail, Al-Qaseem and Riyadh) (Fig. 1). Samples were on average 119 km (10-878 km) from the Red Sea coast (Tohama plain). From each location, seven colonies were sampled. However, we were not able to find and sample native Honeybees from the eastern parts of the country including Alhasa and Aldammam. Samples were preserved in 70% ethanol and were then dissected according to RUTTNER et al. (1978). Body parts were mounted on slides which were then scanned using a high resolution scanner (600 ppi) connected to a desk-top computer system supported with image tool

Table 1. List of morphmetric characters used in this analysis and their numbers as given by RUTTNER.

No.	Character	No.	Character
13+14	Body size	*19:20*	Index of slenderness
5	Length of proboscis	*6*	Length of femur
7	Length of tibia	*8*	Length of metatarsus
9	Width of metatarsus	*10*	Pigmentation of tergite 2
29:30	Cubital index	*9:8*	Metatarsus index
11	Pigmentation of tergite 3	*12*	Pigmentation of tergite 4
13	Longitudinal diameter of tergite 3	*14*	Longitudinal diameter of tergite 4
15	Longitudinal diameter of sternite 3	*16*	Wax mirror, longitudinal
17	Wax mirror, transversal	*19*	Sternite 6, longitudinal
20	Sternite 6, tranversal	*21*	Forewing length
22	Forewing width	*27*	Cubital vein a
28	Cubital vein b	*6+7+8*	Length of hind leg

software (Image tool® 3.0). Basic morphological traits used in this analysis are associated with the Honeybee size and cuticular pigmentations (GOETZE 1964). In total, twenty four morphometric characteristics, reported previously by RUTTNER (1988) as highly discriminatory, were measured (Table 1). Additionally, an empirical series of pigmentation patterns developed by RUTTNER et al. (1978) was used to score colour variation.

Colony sample means were calculated for each character of each bee sample. Data were then analyzed by principal component analysis (factor analysis, rotation method: Varimax with Kaiser Normalization). Subsequently, k-means analysis procedures were performed with an increasing number of clusters, starting from 2 groups. To resolve the number of clusters within Saudi Arabia that best reveal the structure of morphological variation, a goodness-of-fit statistic was calculated for each number of clusters (MEIXNER et al. 2011). Results of factor analysis were then plotted using K-means as group identifier. Subsequently, discriminant analysis using Wilk's lambda was used to verify reallocation probabilities and cluster distances. Analyses were performed using SAS (2006), PASW 18 (2009) and SYSTAT 13 (2009).

Results

In principal component analysis of the 24 morphometic characters for 198 colonies using colony means, the first three factors with eigenvalues >1 accounted for 73.4% of the variance within the population. Factor 1: characters associated with size, factor 2: characters associated with pigmentation, and factor 3: characters associated with indices. Samples were allocated to 3 clusters by K-means clustering, the number of groups which gave highest mean F values for the characters.

Fig. 2 shows the factor scores of the colony means for factor one and factor two, labeled according to the k-means clustering group allocations. K-means clusters occupied different regions in the two-factor space with a moderate degree of overlap at the group margins (Fig. 2), suggesting a reasonable grouping in three clusters.

The group differences were tested by discriminant analysis. Wilks' lamda test demonstrated that differences among the three clusters are highly significant (A= 0.18, F= 26.51; DF= 4.4 and P< 0.0001). In clusters two and three, 100% of the colonies were re-classified correctly, whereas in cluster one 96% of the colonies were re-classified correctly.

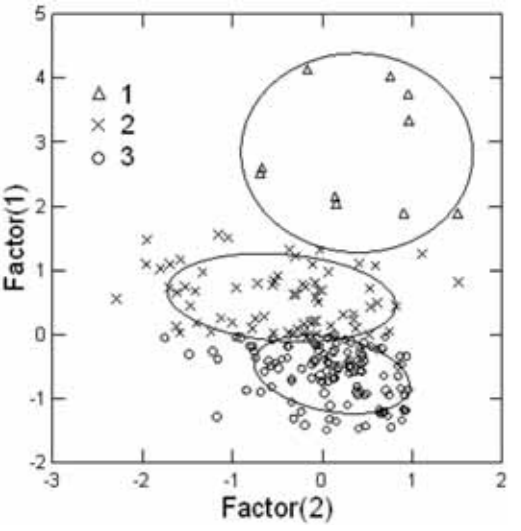

Fig. 2. Principal factor analysis scores plotted using k means groupings and 75% confidence ellipses for the three groups. Factor one is associated with size and factor two with pigmentation. Cluster 1 includes colonies from Tabouk, Hail, Al-Jouf (north). Cluster two includes colonies from Al-Taif, Al-Madinah and Al-Qaseem (central). Cluster three includes of colonies from Asir, Al-Baha, Jazan and Najran (south-west coast).

Mean values for the three clusters are listed in Table 2. Scores of factors 1 & 2 differed significantly among clusters ($P \leq 0.0001$, Kruskal-Wallis test). Pair-wise comparisons between all clusters showed significant differences except for factor 2 ($P \leq 0.0001$, Wilcoxon U test). Means of six main characters listed in Table 2 differed significantly ($P \leq 0.0001$, Kruskal-Wallis test). Similarly, pair-wise comparisons within the six characters showed significant differences except for tergite 4 [colour] ($P \leq 0.01$, Wilcoxon U test).

The geographic positions of the three clusters are displayed in Fig. 3. Although clusters are geographically indistinct and widely overlapping, cluster 3 is predominant in the south (median 18°40'N) with 50% of all cases between 17°20'N and 19°45'N, containing samples from Najran, Jazan, Al-Baha and Asir. Cluster 2 is mainly located in the middle-western region containing samples from Al-Madinah, Al-Taif and Al-Qasseem, which is closer to the central region (median 20°60'N, 50% of all cases between 19°50'N and 21°30'N). The rest of the samples (cluster 1) reside mainly in the northern region from Tabuk, Al-Jouf, Hail, and from Riyadh (central region) (median 25.90°N) with 50% of all cases between 21°40'N and 28°40'N).

A correlation analysis shows a significant association between factor one (characters associated with size) and latitude (N= 198, R= 0.65) (Fig. 4).

The mean of factor one values for samples of cluster two were transitional between cluster one and cluster three (Table 1), indicating significant morphometric variation and a clinal transition of factor one from the north (cluster 1) to the south (cluster 3), with the highest dissimilarities between bees from the far north coast and the far south coast (Table 2). However, no significant correlation to altitude was observed.

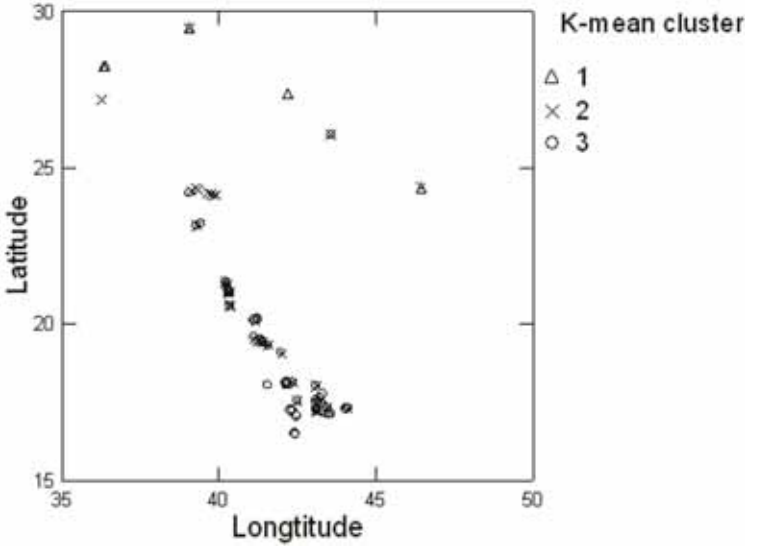

Fig. 3. Geographic distribution of different bee samples included in the principal component analysis (N=198). Each colony from each cluster is represented by a coloured symbol: Green triangles refer to cluster one, red crosses to cluster two and blue dots to cluster three.

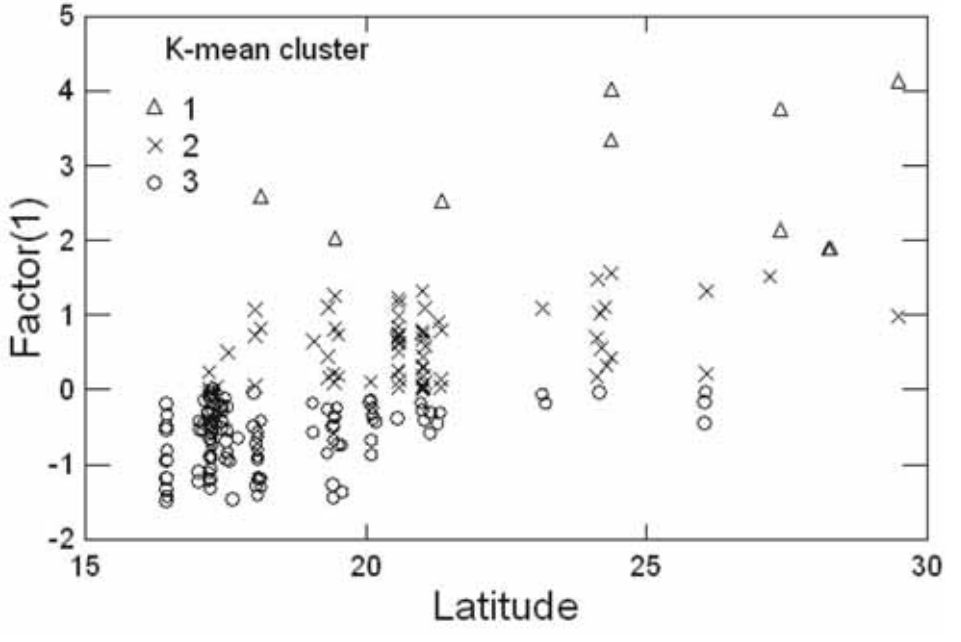

Fig. 4. Relation of Factor one (colony mean) and latitude along the Saudi Red Sea coast. Green triangles: cluster one, red crosses: cluster two, and blue dots: cluster three.

Table 2. Mean characteristics of six morphological traits of local Saudi Honeybees. For comparison, reference data (RUTTNER 1988) were included. Cluster values not statistically different P≤0.01 are marked with *. The table gives mean±STD.

	S. Arabia (cluster 1)	S. Arabia (cluster 2)	S. Arabia (cluster 3)	Significance (clusters)	S. Arabia: Ruttner (1988)
	10	**77**	**111**		**2**
Body size	4.16 ± 0.16	3.69 ± 0.09	3.56 ± 0.08	<0.001	3.82 ± 0.08
Length of hind leg	7.97 ± 0.22	7.46 ± 0.15	7.16 ± 0.14	<0.001	7.01 ± 0.12
Length of forewing	8.79 ± 0.22	8.31 ± 0.19	8.04 ± 0.12	<0.001	8.00 ± 0.19
Width of forewing	3.05 ± 0.22	2.86 ± 0.06	2.76 ± 0.05	<0.001	2.78 ± 0.06
Index of slenderness	87.22 ± 1.79	83.32 ± 2.25	84.28 ± 2.57	<0.001	82.44 ± 1.9
Colour of tergite 4	4.62 ± 0.67 *	5.52 ± 1.11	4.82 ± 0.61 *	<0.001	5.25 ± 1.06
PC Factor 1	2.83±0.89-	0.57±0.45	0.62 ± 0.40	<0.001	
PC Factor 2	0.38±0.74 *	-0.46±0.82	0.10±0.56 *	<0.001	

Discussion

In this study, the morphological characteristics of Saudi bee samples collected from different ecological zones (from the far north, close to the Jordanian border, to the far south near the Yemeni border) were analyzed. Substantial variations were found to confirm that previously described samples (RUTTNER 1988) have been far too low in number to be representative for a large and diverse country such as Saudi Arabia. It was further found that bee samples revealed a clinal transition from north to south, parallel to the Red Sea coast, which is the region where most beekeeping is practiced. Along this line, bees were larger in the north and smaller in the south. Within this gradient, bees could be subdivided best into three main clusters. Cluster 1 included the northern coastal region (Tabouk, Hail and Riyadh), cluster 2 the middle coastal region (from Al-Madinah to Al-Taif), and cluster 3 the southern region (from Al-Baha to the Yemen border). The lightest-coloured bees were in cluster 2, while they were darker in clusters 3 and 1. The darkest samples were collected from locations at high altitude in the southern part near the Yemeni border, which may explain this variation in colour.

Bee samples from the northern regions of Saudi Arabia are possibly more closely related to the Syrian Honeybees, *Apis mellifera syriaca* than to the Yemeni or Omani reference samples. In general, samples were larger in size than previously described by RUTTNER (1978). This region could be a natural distribution limit for the Syrian Honeybees. Cluster two shows an intermediate state of morphometric characters between clusters 1 and 3. Cluster 3 bees are more likely close to *Apis m. jemenitica*. The pattern of variation in Saudi bee populations from the far north to the far south (Tohamah plain) therefore reflects an overlapping and transitional state between the Syrian and the Yemeni Honeybees.

Many of the samples showed a clear morphometric dissimilarity with RUTTNER's reference samples previously described from Saudi Arabia and Yemen (characters associated with size

and colour). Morphometric variation among the clusters of this study exceeds the variation between the Syrian and the Yemeni Honeybee reference samples (RUTTNER 1988). The identification of substantial variation in characters associated with body size and pigmentation among bee samples in this study raises the need to reconsider the allocation of the local bee populations into previously identified ecotypes or subspecies. Furthermore, it might necessitate the description of a new race for the Saudi native bee population. An additional factor to be considered is whether intensive hybridization of the local bee race with other bee races may already have had some impact, particularly in the region of cluster 2. Genetic analysis of the *mt*DNA for different clusters may provide more supportive information.

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